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RESEARCH ARTICLE

Objective assessment of stress levels and health status using routinely measured clinical laboratory parameters as biomarkers

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Abstract
Observed stress intensity was estimated using a scoring system from 0–100. Health status was estimated using the readily available laboratory measurements of C-reactive protein, neutrophil count, and fasting plasma glucose. We found that the stress score determined was linked to patient health status. Further studies are indicated.

Keywords: Stress, ill-health, C-reactive protein, glucose, blood count

Introduction

Stress and health are closely linked. It is well known that responses to short-term stress are generally adaptive and that responses to long-term stress are generally maladaptive. Intense stress arising from highly rigorous competition in present day society has become one of the most common, albeit under-recognized, public health hazards of modern civilization. Because individuals are frequently exposed to exogenous (e.g. environmental stress), endogenous (e.g. oxidative stress), and psychological stress factors, they may develop poor health at any time. The ultimate effect of stress depends on the level of physical responses to stressors. The first step toward understanding stress is to determine the health conditions that stress may cause and the levels of stress that may have an adverse impact on human health. Therefore, it is necessary to quantitatively evaluate the intensity of stress.

The clinical prediction of stress levels and health status is a challenging task. Typically, clinical data are derived from informal, subjective methods, such as a survey questionnaire, which tends to preclude patient-specific diagnoses. The lack of objective information obtained by questionnaires and the length of time required to assess responses leads to infrequent adoption of this approach. Our study was designed to develop a reliable method to take multiple measurements of routinely measured laboratory indices that may be associated with stress. The basic concept underlying the analysis is that the impact of different trait-based stresses on somatic health can be considered as a stress marker to simplify the complex problem of measuring stress. In this study, we established a quantitative system that uses laboratory indices associated with stress and health as biomarkers to objectively assess stress levels and health status and explored the relationship between them. A better understanding of this relationship will be beneficial in developing a theoretical basis for strategies for the prevention and control of stress-related diseases.

Subjects and methods

Subjects

The test group for the assessment of stress consisted of 34 patients (24 males and 10 females) aged 36 ± 8.0 years. All patients had skeletal fractures that were determined from X-ray images. The patients were examined on the morning after the occurrence of the fracture.

Additionally, a total of 10 young adult volunteers (5 females and 5 males) aged between 19 and 23 years were examined at 0700 on day 1 and at 0700 on day 2 to assess their reliability as quality controls. The subjects were accompanied by staff members at all times of day to help them remain relaxed by engaging them in mild

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social activities. Throughout the study period, nutritionally balanced meals (breakfast, lunch, and dinner) were provided to the subjects at appropriate times. The subjects were allowed to sleep normally during night 1, but if a subject was unable to maintain sleep for more than 7 h, as indicated in the protocol, the data were discarded. During day 2 and night 2, subjects were kept awake and examined at 0700 on day 3 as a sleep deprivation group. Subjects engaged in simple activities, such as conversation or playing cards, and remained under continuous observation to prevent them from sleeping, including taking short naps. Subjects understood that they could withdraw from the study at any time.

The test group for poor health status consisted of three subgroups: subjects who were overweight, subjects with stressful occupations, and elderly subjects. The overweight subgroup consisted of 26 subjects (12 men and 14 women) aged between 31 and 80 years with a body mass index of over 27. Subjects wore light clothing and took off their shoes when measuring their heights and weights, which were measured 2 h after dinner with dedicated scales with a precision of 0.1 cm and 0.1 kg, respectively. Doctors and nurses were used to represent the test group with stressful occupations because they experience a relatively high degree of emotional distress (Marshall et al. 2008; Trivedi & Hooke 2008; Allesoe et al. 2010). The occupational stress subgroup consisted of 31 and 18 Chinese doctors and nurses, respectively (13 men and 36 women) aged between 19 and 49 years. Each subject had worked a night shift once or twice per week prior to the study. The elderly subgroup comprised 34 residents of Dalian city, China (16 male and 18 female). Individuals were eligible to participate if they were ≥70 years of age.

Subjects in the poor health status group who met the following criteria were excluded from the study: those suffering from (1) a hematological disorder; (2) cancer, diabetes mellitus, or hepatism; (3) an acute or chronic infection; or (4) a dependence on medication or alcohol.

The test group for disease consisted of 60 patients aged between 44 and 75 years (33 men and 27 women) with different diseases, including 20 cases of cardio-cerebral vascular disease (10 coronary heart disease and 10 cerebral infarction), 20 cases of chronic obstructive pulmonary disease, and 20 cases of cancer (7 gastric cancer, 6 liver cancer, 7 lung cancer). The patients were diagnosed with coronary heart disease according to World Health Organization (WHO) criteria or by coronary angiography (significant coronary artery stenoses ≥50% in at least one major coronary artery) (He et al. 2009). The diagnosis of cerebral infarction was established by clinical evaluation and brain magnetic resonance imaging based on the WHO International Classification of Diseases (Zhang et al. 2009). Chronic obstructive pulmonary disease was defined according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria (Fabbri & Hurd 2003). The FEV_1/FVC, where FEV_1 = forced expiratory volume in 1 s and FVC = forced vital capacity, was <0.7 for all patients. Surgical intervention and pathological findings were used to verify the types of cancer present in patients with cancer.

Thirteen patients with glaucoma (requiring ophthalmologic surgery), 62.4 ± 8.6 years old, 5 male and 8 female, were randomly selected for the glaucoma subgroup; these patients at 4 weeks postoperatively were used as self-controls to observe changes in stress.

A total of 200 normal adults (age 20–49 years, 100 men and 100 women) were selected from a random population as a control group. All of them met the following criteria according to the protocol: free of active infection and symptoms, with no medical history of hepatitis or other serious conditions, including sleep disorders, and no current stressful life events.

The study protocol was approved by the Ethical Committee of Dalian Medical University.

Blood sampling and blood analyses

At 0700, fasting blood samples (5 mL) were drawn from the patient's antecubital vein in two vacuum tubes; one tube contained an anticoagulant and was used for blood cell counts and the other tube contained sodium fluoride and was used to determine blood glucose (Glu) and C-reactive protein (CRP) levels. The tubes containing sodium fluoride were centrifuged for 15 min to obtain serum. After separation, the serum samples were stored at–70°C until further analysis.

At each evaluation, blood was collected in tubes containing anticoagulants, and the neutrophil count [Neut; 1.1~5.9 $(3.5 \pm 1.2) \times 10^9$/L] was determined using an automated hematology analyzer (Sysmex SF-3000; Hyogo,Japan). The serum samples were thawed completely at room temperature and subsequently assayed by laboratory examination in the same run. The Glu level [normal Glu: 3.9–6.1 (5 ± 0.55) mmol/L] was measured using an enzymatic method with an automatic biochemistry analyzer (Hitachi 7170s, Tokyo, Japan). The level of the high-sensitive CRP (HsCRP; <8.2 mg/L) was determined by nephelometry, which involved performing a latex particle-enhanced immunoassay, with a Beckman Access IMMAGE Immunochemistry System. The assay was sensitive enough to detect 0.02 mg/L of serum CRP. The hematologic and biochemical investigations were performed in the clinical laboratory of our University Hospital using standard commercial reagent kits.

Scoring system and statistical analysis

Based on the reference range, we obtained the mean values ($\bar{X}$) and the standard deviation (S) for the normally distributed data (CRP values were first log transformed, –0.54 ± 1.32). The Z-values were calculated according to the following formula, where X is the measured value:

$$Z = (X - \bar{X})/S.$$

The scoring system (0–100) was determined according to the following formula (Hongwei,et al. 2010):

$$Score = \exp(Z-1) \times 100/[1+\exp(Z-1)].$$

The basic principle of the formula is that Z-values are obtained from the measured values to assess the comparability of measured values, and a score between 0 and 100 is subsequently derived from the calculated Z-values. Because Z-value = 1 is considered to be a significant level of stress, when we subtract one from Z, the score will equal 50, which is the most sensitive point for the scoring system.

The overall stress score was equivalent to the mean of the scores of each of the three measured parameters. For example, for scores of Glu, Neut, and CRP of 36, 70, and 45, respectively, the stress score was calculated as follows:

$$\text{Stress score} = (36 + 70 + 45)/3 = 50.3.$$

Receiver operating characteristic (ROC) curves were used to determine the optimal cut-off value (Florkowski 2008). The accuracy (area under the curve), sensitivity (positivity in stress group), and specificity (negativity in control group) were calculated. The positive and negative predictive values were also calculated (Kuk et al. 2010).

The significance of the differences between the original results of the two groups was determined using an independent two-sample *t* test. The significance of the differences was determined using an analysis of variance for the comparison of several results. The significance of the differences in values of the paired parameters was determined by applying the paired two-sample *t* test. The numerical data were examined using the chi-squared test to determine the significance of the differences.

The above analyses were implemented using the SPSS statistical analysis software for Windows. The difference was considered statistically significant when the *p*-value was less than 0.05 (two-tailed test).

Results

The original data for the stress markers for the different test groups are shown in Table 1. Levels of Glu, Neut, and CRP were statistically higher in the stress group than in the control group ($p < 0.05$).

The nine complete paired-data were obtained from the sleep deprivation group on day 1, day 2, and day 3. Experimental data were incomplete for one male subject. The averaged data for all parameters together with the stress score before and after sleep deprivation are shown in Table 2 and Figure 1. No statistically significant differences in the stress scores were noted between day 1 and day 2. The stress scores were higher on day 3 (following sleep deprivation) than on days 1 or 2 ($p < 0.05$).

The data with respect to the association of gender and stress score are summarized in Table 3. The analysis revealed that the stress score was not related to gender ($p > 0.05$).

The comparison of the stress scores among different test groups is shown in Table 4. Stress scores were significantly different in each poor health status subgroup compared with the control group ($p < 0.05$).

The stress scores calculated using these original data are shown in Table 5. ROC analysis between the stress group and the control group shows that the area under the curve (accuracy) was 94.5%, as shown in Figure 2. The data also suggest that the optimal sensitivity/specificity cut-off point for the stress score is 50, as shown in Table 5. The sensitivity (positive rate of the stress group) is 82.5%, the specificity (negative rate of the control group) is 88.5%, the positive predictive value is 87.8%, and the negative predictive value is 83.4%.

Complete paired-data were obtained in the glaucoma group. The stress score was 52.47 ± 15.79 before surgery and 44.80 ± 18.45 postoperatively. Paired analyses showed that there was no significant difference ($p = 0.085$).

Discussion

It has been reported previously that chronic low-grade inflammation, which is identified through CRP levels or neutrophil granulocyte counts, may be involved in stress-related diseases (Singh et al. 2005, Black 2003, Post et al. 2003). Therefore, CRP levels and neutrophil granulocyte counts were selected as the biological markers for the quantification of stress in this study. Moreover, high

Table 1. Original stress marker data for the different test groups.

	Neut (10^9/L)		HsCRP (Ln-transformed)		Glu (mmol/L)	
Groups	$\bar{X} \pm SD$	p	$\bar{X} \pm SD$	p	$\bar{X} \pm SD$	p
Stress	6.04 ± 3.33	<0.0001	1.06 ± 1.68	<0.0001	7.69 ± 1.71	<0.0001
Control	3.67 ± 1.06		−0.13 ± 1.52		5.17 ± 1.20	

Table 2. Averaged data for all parameters assessed before and after sleep deprivation ($\bar{X} \pm SD$).

				p	
Analyte	Day 1 (Control 1)	Day 2 (Control 2)	Day 3 (Sleep deprivation)	D1 vs. D2	D2 vs. D3
Neut (109 /L)	3.50 ± 0.66	3.90 ± 0.47	5.10 ± 1.58	0.047	0.030
Glu (mmol/L)	4.89 ± 0.31	4.77 ± 0.53	4.98 ± 0.26	0.535	0.138
CRPa	−0.38 ± 0.25	−0.41 ± 0.20	−0.42 ± 0.23	0.660	0.831
Stress score	27.23 ± 6.19	28.76 ± 5.59	36.81 ± 10.02	0.590	0.014

[a]Ln-transformed CRP value.

glucose levels are associated with stress; therefore, the fasting plasma glucose level was also selected as a suitable parameter for evaluating the impact of stress on physical health. In this study, the prognostic values of the routine clinical laboratory parameters, HsCRP, neutrophil granulocyte count, and fasting plasma glucose, were all considered to be markers for the comprehensive and quantitative evaluation of stress responses. The direction of change in these variables in response to stress has been reported to be consistent with that observed in the pathological responses. Hence, the score based on these variables is also considered a marker to assess the risk of stress-related diseases. Analyses employed by previous studies have indicated that the responses of these three parameters to stress present a tendency for stochastic independence, and therefore, a scoring system for stress comprising multiple indices was used in this study to avoid bias.

The complete paired stress scores were obtained from adult volunteers, from whom blood samples were drawn twice daily under normal conditions on day 1 and day 2 and after sleep deprivation on day 3. The difference between the scores under normal conditions was not statistically significant ($p > 0.05$). This implies that the reproducibility of this scoring system is satisfactory. As indicated in Figure 1, the stress scores were considerably higher following sleep deprivation than before it. This suggests that the sensibility of this scoring system is sufficient. Our results also indicate that stress score is not related to gender in the normal population, which suggests that the stability of this scoring system is also sufficient.

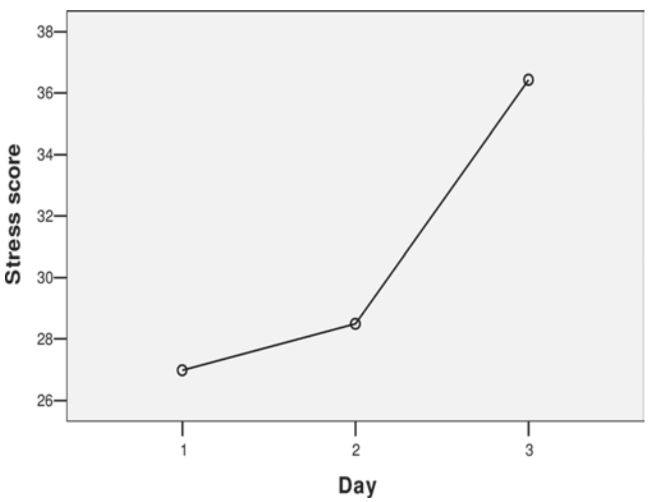

Figure 1. Stress score before (day 1 and day 2) and after (day 3) sleep deprivation to assess the reliability and sensibility of the scoring system.

Table 3. The association of stress score and gender (control population).

Gender	N	$\bar{X}$	SD	p
Female	100	32.91	13.97	0.731
Male	100	33.56	12.54	
Total	200	33.24	13.24	–

Fractures are considered to be ideal models for the quantitative assessment of stress because these stressors are well defined and are capable of influencing certain routine clinical laboratory parameters (Hui et al. 2010). The stress scores of the fracture patients were considerably higher than those of the control subjects, as shown in Table 5. ROC analysis was performed using fractures as stress models for the evaluation of the stress scores to estimate the normal range of the stress score. The results showed that the normal range of the stress score was less than 50, the sensitivity was 82.5%, the specificity was 88.5%, the positive predictive value was 87.8%, and the negative predictive value was 83.4%, indicating that the scoring system may be suitable for the assessment of the stress status of a stressed population.

In this study, we aimed to determine whether the stress score from routinely measured clinical laboratory parameters are associated with different health statuses, to aid in the prevention and control of stress-related diseases. Therefore, subjects who were elderly, overweight, or who had stressful occupations were selected to represent a poor health population to test the response of the stress score. The present results indicate a significant difference between the poor health test group and the control group, suggesting that our quantitative system is effective for determining basic health status.

Cardiovascular and cerebrovascular disease, chronic obstructive pulmonary disease, and cancer have been reported as being responsible for more than 50% of all deaths in China (Department of population and employment statistics national bureau of statistics of China, 2006). Therefore, the test group for disease consisted of individuals with one of these three diseases, to observe the level of stress experienced by the physically ill. Our results show that there is a significant difference between subjects in the disease test group and normal individuals. This suggests that the stress score adopted in this study is sensitive to major illnesses, potentially even in their early stages.

This indicates a clear difference in stress score between low and high stress populations and also between those

Table 4. Comparison of stress scores among different test groups.

Group	$\bar{X} \pm SD$	p	Subgroup	$\bar{X} \pm SD$	p^a	p^b
Poor health	42.49 ± 16.59	<0.0001	Stressful occupation	40.40 ± 16.15	<0.0001	0.045
			Overweight	51.57 ± 15.19		<0.0001
			Elderly	38.54 ± 15.96		0.002
Control	33.24 ± 13.24		Control	33.24 ± 13.24		–

[a]Total comparison.
[b]Comparison with control.

Table 5. Comparison of positive rates among different test groups.

Groups	Stress score ($\bar{X} \pm SD$)	Positive rate (%)		
		Cut-off = 40	Cut-off = 50	Cut-off = 60
Stress	67.29 ± 17.21	100.0	82.4	61.8
Poor health	42.49 ± 16.59	54.1	34.9	19.3
Disease	55.13 ± 16.73	75.0	58.3	45.0
Control	33.24 ± 13.24	28.0	11.5	4.0
p	<0.0001	<0.0001	<0.0001	<0.0001

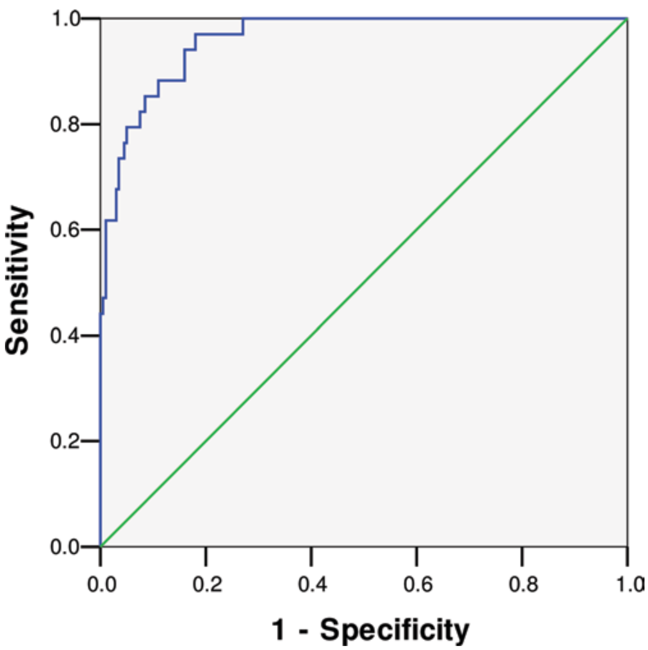

Figure 2. ROC curve for various cut-off levels of stress score (area under the curve = 94.5%).

with healthy and unhealthy statuses. Accordingly, it is potentially possible to predict the degree of the response to stressors and health status in a given individual. It is difficult to estimate the normal range of the stress score. Theoretically, the mean of the normal stress scores should be equal to 27 (Z = 0). Based on our comprehensive analysis, combining the standard deviation values of young healthy individuals (27 + 1.64 × 13.24 = 48.7) and the ROC analysis of stress scores (where the optimal cut-off = 50), we suggest that the lower limit of the normal range of stress score is less than 50. We also suggest dividing basic health status into three phases, indicated by the following ranges of stress scores: a stress score less than 40 represents a healthy phase; 40–50 represents poor health; and more than 50 represents a pre-disease phase, with the likelihood of becoming ill increasing during this phase. It is suggested that people with a poor health status should take appropriate rest and that those with a stress score of more than 50 should undergo medical observation.

The present results indicate that the stress score obtained with the three measured variables (Glu, Neut, and CRP) may potentially be useful as a putative marker for differentiating between levels of stress and the associated link with health. The mechanism by which the expression of these three variables is induced by stress is well established. Hence, the method of obtaining an overall stress score with these variables is likely to be easily accepted by the medical profession. According to their stress scores, patients with glaucoma were in a stressful status before their surgery, with stress scores higher than 50. Those same patients (whose scores were higher than 50) may still be in poor health status 4 weeks after glaucoma surgery, with scores higher than 40. The detailed clinical significance of stress scores increasing or decreasing for individuals with various stress factors or patients with various diseases should be fully determined on various special topics. The results of these studies may assist in identifying novel targets that can be used to create more effective and personalized chronic disease-prevention strategies.

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